

### ABSTRACT OF THE DISCLOSURE

The present invention relates to a method of *ex vivo* amplification of neonatal T cells from umbilical cord blood which comprises obtaining light density mononuclear cells from a sample of umbilical cord blood and then culturing said light density mononuclear cells in a serum-deprived culture medium supplemented with various cytokine combinations. Particularly, there are reported the effects exerted by cytokine combinations including stem cell factor (SCF), interleukin-7, interleukin-4 and interleukin-2, on the amplification of T cells from cord blood mononuclear cells cultured for 10-11 days under serum-deprived conditions. Of all the combinations investigated, SCF plus interleukin-7 sustained the best fold increase (FI) of total nucleated cells ( $FI=6.4\pm1.17$ ), amplifying preferentially  $CD4^+$  over  $CD8^+$  T cell subsets ( $FI=4.72\pm0.79$  vs  $2.73\pm1.2$ , respectively,  $p<0.05$ ). The addition of interleukin-2 to this combination did not significantly increase the total number of cells generated ( $FI=7.4\pm2.27$ ) but allowed preferential amplification of  $CD8^+$  over  $CD4^+$  T cells ( $FI=6.04\pm0.14$  vs  $1.67\pm0.6$ , respectively,  $p<0.05$ ). Single strand conformation polymorphism analysis of the T-cell receptor  $V_\beta$ -chain rearrangements expressed by the expanded T cells indicated that the complexity of the T-cell repertoire had increased after 10 days of culture in the presence of SCF and IL-7. Interestingly, a modest expansion ( $FI=8.67\pm1.5$ ) of myeloid progenitor cells was also observed in these cultures. These results indicate that it is possible, by modulating the cytokines added to the cultures, to expand specific T cell subsets for adoptive immunotherapy without losing myeloid progenitor cells necessary for neutrophil recovery after cord blood transplantation.